MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2022

April–June 2022—Volume 137, pp. 335–344

https://doi.org/10.5248/137.335

First record of *Lactocollybia variicystis* from Asia

Aiman Izhar*, Muhammad Usman, Hira Bashir, Shazia Ashraf, Abdul Rehman Niazi, Maria Latif & Abdul Nasir Khalid

Fungal Biology and Systematics Research Laboratory, Institute of Botany, University of the Punjab, Quaid-e-Azam Campus-54590, Lahore, Pakistan

* Correspondence to: *aimanizhar25@gmail.com*

Abstract—During surveys of macrofungi in Pakistan, eight specimens belonging to the genus *Lactocollybia* were collected from the plains of Punjab and foothills of Murree. Morphological studies and molecular characterization refer all collections to a single species, *Lactocollybia variicystis*, previously restricted to Africa and Europe; this is the first report from Asia. Except for geography, no significant differences were found among African, European, and Pakistani collections.

Key words—*Agaricales*, gloeocystidia, *Gymnopus*, macrocystidia, taxonomy

Introduction

Lactocollybia Singer is represented by 20 species (www. Indexfungorum.com, accessed October 2020) that are mostly tropical (some subtropical) with a few species reported from South and North America (Singer 1986, Senthilarasu & Kumaresan 2016). *Lactocollybia* accommodates species that are collybioid or (less frequently) pleurotoid and characterized by mostly pure white carpophores, slightly to non-hygrophanous pilei, stipes that are mostly distinct (but may be reduced to absent) and lack annuli, latex that is mostly present, and with a white to creamy spore print. Anatomically, these saprophytic fungi produce basidiospores that are thin-walled and inamyloid and tissues that frequently possess gloeovessels or gloeocystidia throughout. Most species are lignicolous, but others fruit on soil, humus, and bark of

living trees. Morphologically, *Lactocollybia* resembles *Macrocystidia* in its abundant gloeocystidia, frequent cystidia, and inamyloid basidiospores, but the genus is separated by its white spore print and acyanophilic basidiospore walls (Singer 1986). Among white-spored mushrooms, *Gymnopus* (Pers.) Roussel, similar in form to *Lactocollybia*, differs in its irregularly arranged coralloid elements, present on the edge of the pileipellis (Antonín & al. 2013).

Lactocollybia is divided into five small sections based on the basidiome structure (collybioid, mycenoid, omphalioid, or pleurotoid), pigmentation, subhymenial structure; the presence or absence of latex, clamp connections, and gloeocystidia; and habit on living tree stumps, bark, or fallen fruit (Singer 1970, 1986; Hosen & al. 2016; Senthilarasu & Kumaresan 2016). *Lactocollybia variicystis* is referred to *L.* sect. *Albae* Singer based on its basidiomata lacking a veil and latex production, the presence of clamp connections and numerous gloeocystidia, and a lignicolous habit. Only a single species has previously been reported from Pakistan: *Lactocollybia angiospermarum* Singer [= *L. epia* (Berk. & Broome) Pegler] (from districts Lahore, Narowal, and Sargodha on bark of *Ziziphus jujuba* Mill. and *Dalbergia sissoo* DC.; Ahmad 1980).

Molecular phylogenetic analyses for the genus have not yet been completed, and only a few sequences of the nrITS (nuclear ribosomal internal transcribed spacer) region and partial sequences of the nuclear large subunit (nrLSU) are available at NCBI. Here we generated eight nrITS sequences from basidiomata of *Lactocollybia variicystis* collected from Pakistan during 2016–18. The species was originally described from South Africa, (Reid & Eicker 1998) and later reported from Spain (Menorca, with a Mediterranean climate) and equatorial west Africa (São Tomé, with a tropical maritime climate) (Salom & Siquier 2014; Desjardin & Perry 2017; Chou & al. 2020). This report of its occurrence (from a subtropical dry climate on loamy soil of Pakistan) represents the fourth worldwide and the first from Asia.

Material & methods

Morphology

Fresh samples were collected in the districts of Gujrat, Hafizabad, Islamabad, and Sheikhupura (all in Punjab, Pakistan) and photographed in natural light. Basidiomata were dried using a fan heater. Macroscopic characters were described from fresh material and field photographs, and microscopic features were obtained by reviving hand-sections of dried basidiomata for examination under a light microscope. Mountants used were 5% KOH, Melzer's reagent (for basidiospore amyloidy) and 1% Congo red (for staining gill tissues). Sections were studied at magnifications ≤1000×. Color terms follow Munsell (1975). A minimum 20 basidiospores, basidia,

cheilocystidia, pleurocystidia, pileus and stipe elements from each collection were measured using ScopeImage 9.0(X5). Abbreviations include avl = average length, avw = average width, Q = basidiospore length/width ratio, and avQ = mean Q of all basidiospores. All collections were deposited in the Herbarium, Institute of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Molecular studies

Approximately 2 mg of fungal tissue was taken from dried specimens to extract DNA following a modified CTAB protocol (Bruns 1995). The universal fungal primer pairs ITS1F (Gardes & Bruns 1993) and ITS4 (White & al. 1990) were used to amplify the ITS region following the PCR procedure of 1 min denaturation at 94 °C; then 35 cycles for 30 sec, 1 minute at 50 °C, and 1.5 min at 72 °C; ending with a final elongation for 1 min at 72 °C. The ITS amplicons were sequenced by TsingKe (Beijing, China).

Sequences were compared using the BLAST tool available on the NCBI database. For phylogenetic reconstruction, BLAST searches were executed to find sequences showing maximum identity, and those referred by Hosen & al. (2016) were retrieved from GenBank. The multiple sequences were aligned using MUSCLE (Edgar 2004), then manually adjusted in BioEdit (Hall 1999). A phylogenetic tree using Maximum

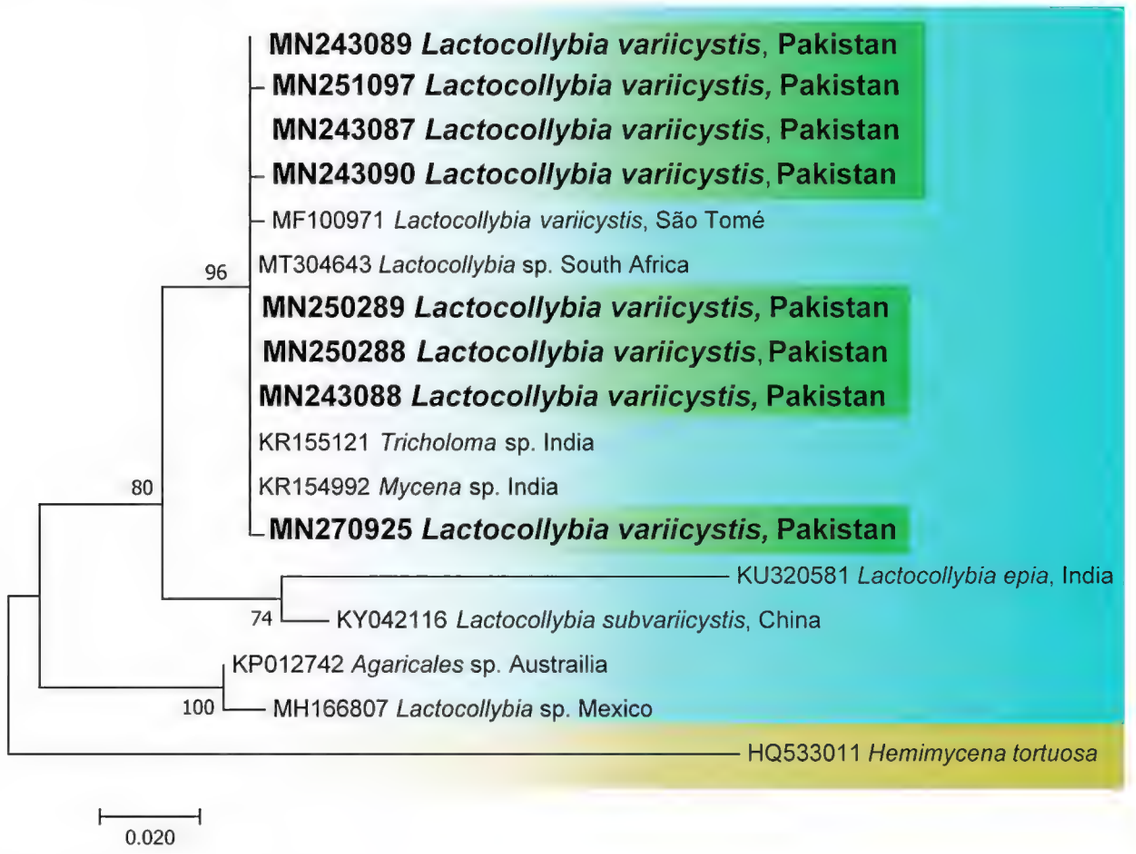

Fig. 1. Phylogram of *Lactocollybia variicystis* obtained by Maximum Likelihood method based on ITS sequences. Bootstrap values ≥70% are given above or below the branches. Species collected from Pakistan are highlighted in bold.

Likelihood criteria and the Neighbor-Joining method was constructed in MEGA7 using Jukes-Cantor model under 1000 bootstrap replicates (Jukes & Cantor 1969, Kumar & al. 2016). Clades with a BS value >50% were regarded as highly supported (Hillis & Bull 1993). This aligned sequence data matrix was exported to Adobe illustrator CS v.3 for editing.

Molecular phylogenetic results

The nrDNA ITS sequences from all collected *L. variicystis* basidiocarps measured 623–638 bp. In BLAST, the Pakistani *L. variicystis* sequences showed 99% similarity with a specimen from India labelled as *Mycena* sp. (KR154992) and 98% similarity with *Lactocollybia variicystis* (MF100971) from São Tomé. Sequences included in the final analysis represented the outgroup *Hemimycena tortuosa* (HQ533011), different *Lactocollybia* species, and a few closely related BLAST sequences. The resulting phylogeny nested all Pakistani *L. variicystis* sequences in the same clade as previously reported *Lactocollybia* species (Fig. 1).

Taxonomy

Lactocollybia variicystis D.A. Reid & Eicker,
Mycotaxon 66: 159. 1998. Figs 2, 3

Basidiomata small, dry, ≤2.7–3.0 cm. Pileus 1.4–2.9 cm, avw = 1.9 cm diameter, reniform when immature, broadly convex to plano-convex at maturity, slightly depressed at disc, margins plicate-sulcate striate, surface smooth, white (N10), pale yellow when bruised. Lamellae light pink (5YR8/8), adnate, horizontal, close to subdistant, crisped, edges even, lamellulae in 1–2 tiers, wavy. Stipe 1.0–1.5 × 0.5–0.8 cm, central to eccentric, almost equal, rarely tapered towards base, hollow; dry, minutely pruinose, white to pale yellow when old. Odor cabbage-like. Taste not recorded.

Basidiospores (6.4–)7.4–9.4(–9.8) × (4.8–)5.2–5.8(–6.2) µm, avl × avw = 7.9 × 5.4 µm, Q = 1.4–1.6, avQ = 1.5, broadly ellipsoid to ellipsoid, some amygdaliform, thin-walled, smooth, apiculate, multi-guttulate, inamyloid, hyaline in KOH. Basidia 22.5–29.5 × 6.5–9.5 µm, avl × avw = 23.8 × 7.8 µm, thin-walled, hyaline in KOH, narrowly clavate, 2–4-spored, guttulate. Cheilocystidia 47.5–76.5 × 6.5–15.5 µm. avl × avw = 55.6 × 11.7 µm, frequent, thin-walled, hyaline in KOH, narrowly cylindrical to fusoid, ventricose, sometimes lageniform, capitate, inamyloid. Gloeocystidia 53–105 × 9–23 µm, clavate, some ventricose with obtuse apex, omnipresent throughout gills. Pleurocystidia 49–79 × 6.5–16 µm, avl × avw = 56 × 10.5 µm, frequent, thin-walled, hyaline in KOH, narrowly cylindrical, versiform,

Fig. 2. *Lactocollybia variicystis*: basidiomata: A, B = LAH35712: C = LAH36360: D, F = LAH35348: E = LAH35347: G = LAH35346. Scale bars = 1 cm.

cylindrically capitate, inamyloid. Pileipellis with cutis, hyphae (2.5–5 µm), radially arranged with elements of gloeosystem interspersed, later appearing as fusoid intercalary swellings, hyaline in KOH, inamyloid; gloeocystidia

110–132 × 5–14 µm, narrowly clavate, versiform; loosely interwoven. Stipitipellis a cutis; hyphae 2.5–8.2 µm broad, avw = 5.1 µm, irregularly arranged, hyaline in KOH; gloeocystidia 88–120 × 9.7–16.9 µm, with yellowish golden refractive contents, variable intercalary swellings present. Clamp connections frequent.

Habitat & distribution—Gregariously growing on bark of *Psidium guajava* L. and *Vachellia nilotica* (L.) P.J.H. Hurter & Mabb. Previously reported from Africa and Europe, and now for the first time from Asia.

Specimens examined—**PAKISTAN. Punjab: Sheikhupura district**, 31.7111°N 73.9878°E, elevation 236 m a.s.l., gregarious on tree bark of *Psidium guajava,* 16 July 2017, Aiman Izhar SKP12_2 (LAH 35712; GenBank: MN250288); 1 August 2018, Aiman Izhar SKP12_1 (LAH 36360; GenBank: MN250289). **Gujrat district**, Pabbi Forest Park, 32.8315°N 73.8360°E, elevation 286 m a.s.l., gregarious on the bark of *Vachellia nilotica*, 11 August 2016, Muhammad Usman & Abdul Nasir Khalid MU14 (LAH 35346; GenBank: MN243087); 25 August 2016 MU15 (LAH 35347; GenBank MN243088); 1 August 2017, MU26 (LAH 35348; GenBank: MN243089); 5 August 2018, MU55 (LAH 35349; GenBank: MN243090). **Islamabad district**, 33.7167°N 72.9167°E, elevation 1604 m a.s.l., on bark, 17 July 2017, Shazia Ashraf MH9 (LAH 36396; GenBank: MN251097). **Hafizabad district**, 32.3333°N 73.7667°E, elevation 207 m a.s.l., on bark, 30 July 2017, Muhammad Ali HZ-54 (LAH35808; GenBank: MN270925).

Discussion

Our phylogenetic analysis grouped all nrITS sequences generated from Pakistani *Lactocollybia* collections (MN250288, MN250289, MN243087, MN243088, MN243089, MN243090, MN251097, MN270925) with *L. variicystis* (MF100971) from São Tomé, *Tricholoma* sp. (KR155121), and *Mycena* sp. (KR154992) from India.

Until now few *Lactocollybia* species have been molecularly analyzed. Our study includes eight Pakistani collections from four sampling sites. Two collections (SKP12_2 and SKP 12_1) were on the bark of *Psidium guajava* from Sheikhupura, a subtropical to dry, industrial area of Punjab subject to extreme climate variations and heavy rainfall during the monsoon season from mid-June to August. The average rainfall is 630 mm and the area has mixed loamy-textured soil (Shaheen & al. 2019). The district flora is dominated by *Senegalia modesta* (Wall.) P.J.H. Hurter, *V. nilotica*, *Prosopis cineraria* (L.) Druce, and *Tamarix aphylla* (L.) H. Karst. (Rahim & al. 1975, Izhar & al. 2019).

Four collections (MU-14, MU-15, MU-26, and MU-55) were made from Pabbi Forest Park (Gujrat district, a portion of Himalayan foothills) spread

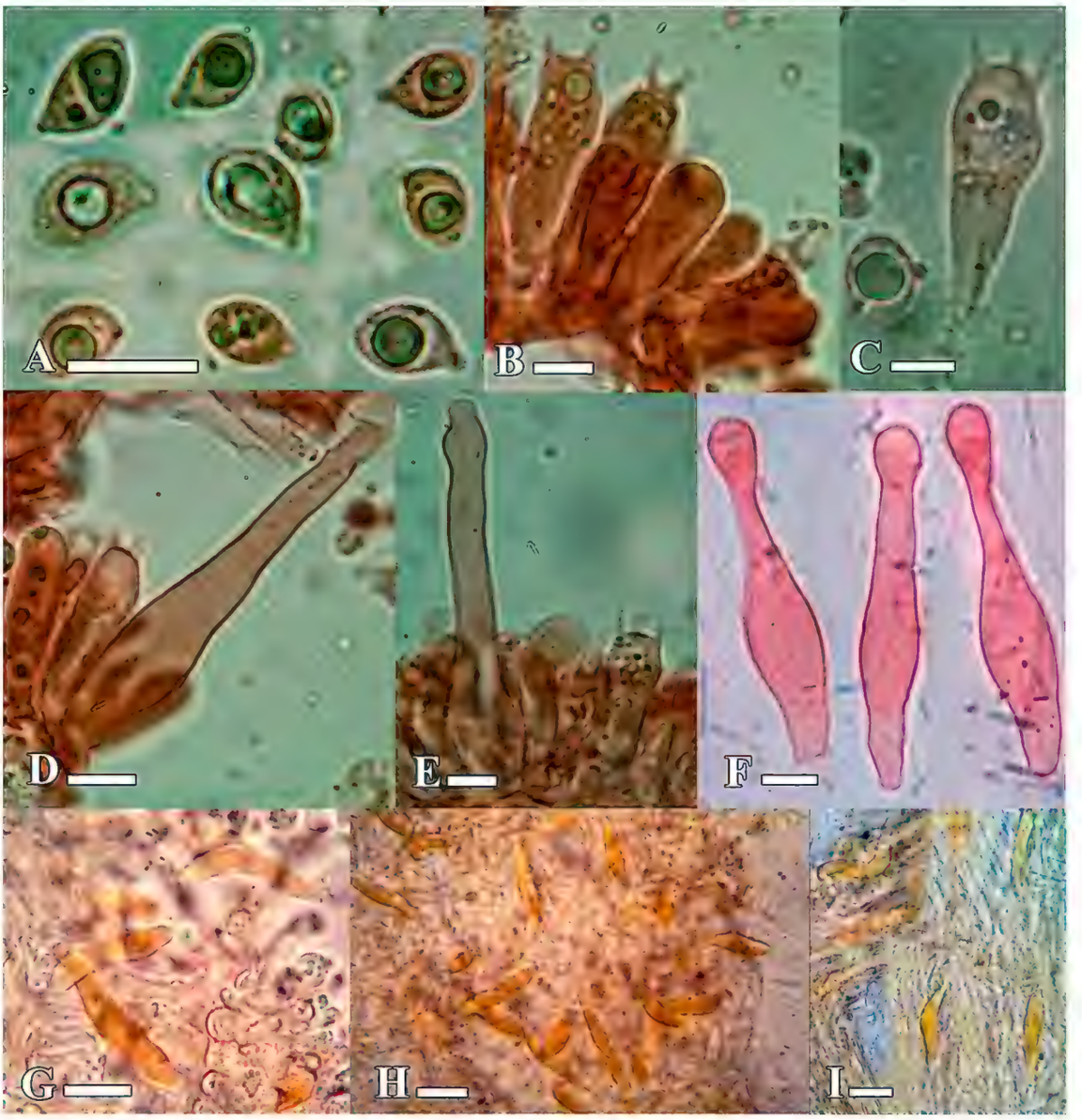

Fig. 3. *Lactocollybia variicystis* (LAH 35712, LAH 36360). A. basidiospores; B, C. basidia; D, E. cheilocystidia; F. pleurocystidia; G. gloeocystidia in lamellae; H. gloeocystidia in pileus; I: gloeo-elements in stipe. Scale bars: A = 10 µm; B, C = 20 µm; D–F = 10 µm; G–I = 30 µm.

almost over 160 km^2 dominated by *S. modesta*, *Grewia tenax* (Forssk.) Fiori, and *Prosopis juliflora* (Sw.) DC and with an annual rainfall of 774 mm per year (Usman & Khalid 2018). The basidiomata occurred gregariously on bark at the base of *Vachellia nilotica*. One collection (HZ-54) was from district Hafizabad with a semi-arid climate and an annual rainfall of 50–70 mm (Umair & al. 2017). The last collection (MH9) is from Margalla hills, (Islamabad), having a humid subtropical climate and a dominant vegetation including *Senegalia modesta* and *V. nilotica*.

The Pakistani *L. variicystis* collections share a remarkable similarity with the African type material from a *Salix* stump in a humid subtropical climate (Reid & Eicker (1998). The South African specimen contrasted with our collection by its slightly narrower (≤1.8 cm) pileus, longer and thinner stipe (maximum 3.2 × 0.2 cm), shorter basidiospores (max length = 8 µm), smaller cheilocystidia (avl × avw = 17–40 × 5–11 µm), and pleurocystidia resembling gloeocystidia.

The Pakistani *L. variicystis* has a pileus slightly depressed at the center, in contrast to the broadly umbonate pilei of the São Tomé specimens of *L. variicystis* collected from decaying banana and cacao plants in a hot, humid, tropical maritime climate (Desjardin & Perry 2017). Other features shared by the Pakistani and São Tomé collections are golden gloeosystem elements with central swellings and narrow edges scattered throughout the pileus and stipe. Our collections differ in their wider (avw = 1.9 cm) pilei, which are significantly smaller (0.6 cm) in European collections. Basidiospores from Pakistani collections are relatively broad (4.8–6.2 µm) compared to those in European collections (3.5–5.2 µm; Salom & Siquier 2014). Phylogenetically, there were four differences between the Pakistani and São Tomé DNA sequences (at the 64, 197, 225, and 249 bp positions) but, except for the two or three minor differences mentioned above, no significant morphological differences have been observed, supporting identification of the Pakistani specimens as *L. variicystis.*

The similar *Lactocollybia epia* (KU320581) and *L. variicystis* are both characterized by a convex to plane pileus with a depressed center and the same basidiospore spore size (6–9 × 4–6 µm) in the descriptions given by Corner (1994) and Yang (2000). However, subsequent reports clearly separate *L. epia* by its larger basidiomata with a 1–5 cm broad pileus and stipe measuring 2–8 × 0.2–0.5 cm, much more elongated and thinner basidiospores (7.5–11 × 4.2–5 µm), and cylindrical to clavate cheilocystidia (Pegler 1977, 1986; Cortez & Sulzbacher 2009).

Our phylogenetic analysis places *L. variicystis* closer to *L. subvariicystis* Hosen & T.H. Li (first described from China) in the same clade. Both species share a subtropical habitat, small white basidiomata, gloeosystemic elements in pileus and stipe, and frequent clamp connections. Contrasting features include in *L. subvariicystis* the presence of adnate to sinuate lamellae, long narrow basidiospores (8–10.5 × 4.5–5.3 µm), scarcely plicate margins, and association with *Acacia confusa* Merr. (Hosen & al. 2016).

Acknowledgements

The authors are grateful to Higher Education Commission, Pakistan, for financial assistance under National Research Program for Universities (project no. 20-3383/NRPU/R&D/HEC/14/184). We are sincerely obliged to Dr. Arun Kumar Dutta, (Department of Botany, West Bengal State University, Barasat, India) and Dr. Aamna Ishaq (Department of Biological Sciences, University of Veterinary & Animal Sciences (UVAS), Ravi Campus, Pakistan) for their peer review and valuable comments on this manuscript for Mycotaxon. We also obliged to Dr. Munazza Kiran (Fungal Biology and Systematics Research Laboratory, Institute of Botany, University of the Punjab, Lahore, Pakistan) for initially reviewing and commenting on this research article. Special thanks are due to Dr. Amy Y. Rossman (Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, USA) for linguistic revision of the manuscript.

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